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Evaluation of the ability of barley genotypes containing different amounts of β-glucan to alter growth and disease resistance of rainbow trout *Oncorhynchus mykiss*

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Abstract

A feeding trial was performed to screen three barley genotypes containing different levels of β-glucan for their ability to influence growth, immune function, and disease resistance of rainbow trout. Three experimental diets were prepared by substituting each of three barley genotypes containing different amounts of β-glucan, low (38 g/kg), average (52 g/kg) and high (82 g/kg) β-glucan barley, respectively, for the entire wheat portion (321 g/kg) of the diet. An additional test diet which consisted of the control diet supplemented with a commercially available yeast β-glucan product (MacroGard) at the manufacturer's recommended level of 2 g/kg also was evaluated. Juvenile rainbow trout (approximately 14.3 g initial weight) cultured in 145 L fiberglass tanks (three tanks/diet; 50 fish/tank) in a fresh water flow through system were fed the test diets by hand to satiation for 9 weeks. At 3 and 9 weeks post weighing, fish were sampled for determination of respiratory burst activity, and lysozyme and TNF-α expression. At the conclusion of the feeding trial, fish remaining after sampling were pooled by diet; one subsample was examined for their ability to respond humorally to infectious

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hematopoietic necrosis virus and two other subsamples were challenged by intraperitoneal injection with a virulent strain of the same virus. Substituting barley flour for wheat flour in a fish-meal based diet did not substantially alter weight gain or proximate composition of rainbow trout. In contrast, positive effects of barley genotype on disease resistance were observed. Trout fed the average or high β-glucan barley genotypes had survival rates similar to those fed the commercially available yeast β-glucan supplemented diet and higher than trout fed the wheat control diet following viral challenge. The results indicate that select barley genotypes can be substituted for wheat without significant detrimental effects on production efficiency while potentially increasing resistance to viral pathogens.

Keywords: Barley genotypes; β-Glucan; Rainbow trout; Immune function

1. Introduction

As aquaculture strives to maintain profitability and sustainability in an era of escalating competition for fishmeal and fish oil, plant-based feedstuffs are being incorporated into fish feeds at an increasing rate. Concomitant with the higher inclusion levels and increased variety of plant-based ingredients in these feeds is a pressing need to understand how quantitatively minor, yet biologically active, plant components alter fish growth and health. Also lacking is information regarding how levels of these bio-active components differ between plant sources. This information is also necessary to avoid substitutions which negatively impact production.

In the United States wheat has traditionally been the most utilized carbohydrate source in commercial salmonid diets. However, other cereal grains, including barley, also may be suitable alternatives for inclusion in rainbow trout diets. Barley has the added potential of improving fish health due to the high β-glucan content. β-Glucans are structural components of the cell wall of bacteria, fungi, yeast and some plants. In contrast to β-glucans from fungi and yeast which contain β 1,6 and β 1,3 linkages, β-glucans from plants such as barley, oats, rye and wheat primarily consist of mixed β 1,3 and β 1,4 linkages at average levels of 50, 50, 20, and less than 10 g/kg, respectively. Few studies have accounted for endogenous levels of β-glucans in plant-based aquatic animal feedstuffs. This oversight is due, in part, to the fact that analytical methodologies for quantification of β-glucans, are only now becoming widely available and, in part, to the contention that the immunostimulation potential of β-glucans present in most feedstuffs would be minimal due to an inability of these endogenous glucans to freely bind glucan receptors and thus activate intestinal macrophages (Czop, 1986; Goldman, 1988).

Identifying varieties of cereal grains suitable for utilization in fish feeds offers potential for more flexible, and possibly more economical, formulations for feed manufactures and new markets for grain producers. However, there are no clearly defined parameters for determining the suitability of a particular genotype of barley for use in fish feed. In the present study, a feeding trial was performed to screen three barley genotypes which differed in β-glucan content for their ability to influence growth, immune function and disease resistance when barley was substituted for the wheat portion of a fish meal-based diet.

2. Materials and methods

2.1. Experimental design and diets

A practical-type control diet that met or exceeded all the known dietary requirements of rainbow trout was formulated (National Research Council, 1993). The diet was formulated to contain approximately 410 g/kg crude protein and 150 g/kg crude fat with approximately half of the protein from fish meal and half from plant sources with wheat flour as the primary carbohydrate source (Table 1). Three hull-less barley genotypes including 01HR 5651, 01HR 2822 and 02HR 1808 with β-glucan contents at 38 g/kg (low; 01HR 5651), 52 g/kg (average; 01HR 2822) and 82 g/kg (high; 02HR 1808), respectively, were obtained from the USDA-ARS National Small Grains Germplasm Research Facility (Aberdeen, ID, USA). Experimental diets were prepared by substituting each of three barley genotypes for the entire wheat flour portion of the diet. An additional test diet was evaluated which consisted of the control diet supplemented with a commercially available yeast β-glucan

Table 1
Ingredients and proximate composition (g/kg) of experimental diets on an as fed basis[a]

Ingredient	Diets	
	Wheat	Barley
Herring meal	344	344
Corn gluten meal	72	72
Soybean meal	154	154
Wheat flour	321	–
Barley flour	–	321
Fish oil	78.8	78.8
Lecithin	20	20
Vitamin premix[b]	4	4
Choline chloride	5	5
Vitamin C	0.2	0.2
Trace mineral mix[c]	1	1
Analysed composition[d]		
Moisture	40.0	33.3
Gross energy (MJ/kg)	24,196	24,129
Crude protein	462.9	454.6
Ether extract	135.3	133.9
Ash	36.7	37.9

[a] Dietary β-glucan content was calculated based on reported values of 10 g/kg β-glucan content of wheat, 680 g/kg β-glucan content of MacroGard and analysed values of 38 g/kg (low), 52 g/kg (average) and 82 g/kg (high) β-glucan barley to be 3.2 g/kg for the wheat diet, 4.5 g/kg for the wheat diet supplemented with 2 g/kg MacroGard, and 12.2, 16.7 and 26.4 g/kg for the low, medium, and high β-glucan barley diets, respectively.

[b] Provided the following per kg diet: thiamin mononitrate, 62 mg; riboflavin, 71 mg; niacin, 294 mg; calcium pantothenate, 153 mg; pyridoxine hydrochloride, 50 mg; folic acid, 22 mg; vitamin B_{12}, 0.08 mg; D-biotin, 0.8 mg; myoinositol, 176 mg; retinol acetate, 8818 IU; vitamin D_3, 588 mg; α-tocopherol acetate, 670 mg; menadione sodium bisulfite complex, 37 mg.

[c] Provided the following per kg diet: KI, 1.5 mg; $MnSO_4 \cdot 7H_2O$, 75 mg; Na_2SeO_3, 2 mg; $CoCl_3 \cdot 6H_2O$, 1.0 mg; $CuSO_4 \cdot 5H_2O$, 3 mg; $FeSO_4 \cdot 7H_2O$, 50 mg.

[d] $N = 2$ samples per diet.

product (MacroGard, Immunocorp AS, Oslo, Norway) at the manufacturer's recommended level (2 g/kg) of the diet (Table 1).

Prior to mixing, all ingredients were ground using an air-swept pulverizer (Jacobsen 18H, Minneapolis, MN, USA). Dry ingredients were mixed in a horizontal paddle mixer (Marion Mixers, Marion, IA, USA) and a portion (approximately 1/3) of the added oil was mixed into the dry ingredients along with the lecithin. The mash was then extruded through a 3.0 mm die of a Buhler twin-screw cooking extruder (DNDL-44, Buhler AG, Uzwil, Switzerland). Barrel temperature averaged 127 °C and die pressure was 410 psi, with an estimated barrel residence time of 18 s. The diets were dried in a pulse bed drier extruder (Buhler AG, Uzwil, Switzerland) with product temperature remaining below 65 °C and final moisture content less than 80 g/kg. After the diets were dried, the remaining oil was applied with a vacuum-coater (Phlauer, Minneapolis, MN, USA).

2.2. Experimental animals, feeding and sample collection

A domesticated strain of juvenile rainbow trout (House Creek strain, College of Southern Idaho, Twin Falls, ID, USA) with an average initial weight of 14.3 ± 0.2 g was utilized. Fish were fed all they would consume in 30 min to approximate satiation 3 times per day 6 days per week. Fish were fed for 9 weeks with three replicate tanks per diet (50 fish/tank). Trout were reared in 150 l fiberglass tanks with 4–6 l/min of constant temperature (14.8 °C) spring water supplied by gravity. Rainbow trout were bulk-weighed and counted every 3 weeks, and fish growth rates and feed conversion ratios were calculated. Prior to the start (five fish), at 3 weeks (three fish/tank) and at the end of the feeding trial (three fish/tank) were sampled for determination of proximate composition. All fish handling and experimental protocols were approved by and conducted in accordance with the guidelines of the University of Idaho's Animal Use and Care Committee.

2.3. Proximate composition analyses

Dry matter (934.01) and ash (942.05) analysis of fish and diets was performed according to standard methods (AOAC, 1995). Crude protein (990.03) was determined by the Dumas method (AOAC, 1995) on a LECO nitrogen analyzer (FP428, LECO Corporation, St. Joseph, MI, USA). Lipid was determined using a Foss Tecator Soxtec HT Solvent Extractor, Model Soxtec HT6 (Höganäs, Sweden). Gross energy was determined using an adiabatic bomb calorimetry (Parr 1281, Parr Instrument Company Inc., Moline, IL, USA). β-Glucan content of barley included in the experimental diets was determined with a mixed-linkage assay (Megazyme International Ltd., Wicklow, Ireland).

2.4. Respiratory burst response

To assess the nonspecific immune responses, three fish were randomly sampled from each tank at 3 and 9 weeks following feeding. Fish were killed and the anterior kidney and spleen of test animals was removed. Tissues were pooled by tank and used to isolate phagocytic cells for respiratory burst activity (Secombes, 1990) with minor modifications as described by Sealey and Gatlin (2002).

2.5. RNA isolation and immune gene expression

To assess immune gene expression, head kidney samples from three additional fish per tank were isolated at 3 weeks and at the end of the feeding trial (9 weeks) for RNA extraction to examine the inflammatory response markers, *lysozyme* and *TNF-α* (Overturf et al., 2003) by real time RT/PCR. Isolated tissue was immediately placed into a microtube containing TRIZol (Invitrogen, Carlsbad, CA, USA) and isolated according to the manufacturer's protocol and then quantified. Real time RT-PCR was carried out using an ABI Prism 7900HT Sequence Detection System and the TaqMan One-Step RT-PCR Master Mix Reagents kit from ABI, according to the protocol provided by ABI (Foster City, CA, USA). Real time quantitative RT-PCR was carried out using an ABI Prism 7900HT Sequence Detection System and the TaqMan One-Step RT-PCR Master Mix Reagents kit from ABI, according to the protocol provided by ABI (Foster City, CA, USA). The final concentration of each reaction was: Master Mix, 1× (contains AmpliTaq Gold enzyme, dNTPs including dUTP, a passive reference, and buffer components); MultiScribe reverse transcriptase, 0.25 U/μl; RNase inhibitor mix, 0.4 U/μl; total RNA, 100 ng; forward and reverse primers, 900 nM for *TNF-α* and *β-actin*, 300 nM for *lysozyme*; and probe, 250 nM for *TNF-α* and *β-actin*, 200 nM for *lysozyme*. For *TNF-α* and *β-actin*, primers and probe were designed by ABI's Assay by Design service. For *lysozyme*, primers and probe were designed using Primer Express software (ABI; Foster City, CA, USA). GenbankAccession number and primer/probe sequence (listed 5′–3′) for *β-actin*: AF254414, BactinF: CCCTCTTCCAGCCCTCCTT, BactinR: AGTTGTAGGTGGTCTCGTGGATA, BactinMGB: 6FAM-CCGCAAGACTCCATACCGA-NFQ; *lysozyme*: AF452171, Lys131F: TGGGTTTGCCTGTCAAAATG, Lys227R: TGTTGATCTGGAAGATGCCATAGT, LysT: 6FAM-TCG AGCTACAATACCCAGGCCACCA-TAMRA and *TNF-α*: AJ401377, TNFF: TGGAGCCTCAGCTGGAGATATT, TNFR: CCGGCAATCTGCTTCAATGTATT, TNFMGB: 6FAM-CATTGGTGCAAAAGATAC-NFQ. Cycling conditions for *TNF-α* and *β-actin* were as follows: 30 min at 48 °C, 10 min at 95 °C, then 40 cycles of PCR consisting of 15 s at 95 °C followed by 1 min at 60 °C. Cycling conditions for *lysozyme* were: 2 min at 50 °C, 30 min at 60 °C, 5 min at 95 °C, then 40 cycles of PCR consisting of 20 s at 95 °C followed by 1 min at 62 °C. Assays were run in duplicate on RNA samples isolated from individual fish. A serial dilution of six duplicate standards was run with each primer/probe set for quantification. As a cellular mRNA control, *β-actin* levels were determined for each sample and used in the normalization of specific expression data (Kreuzer et al., 1999). The fluorescence output for each cycle of the polymerase reaction was measured and downloaded to a PC computer upon the completion of the entire run. Accumulated data was analysed using the computer program Sequence Detector version 2.1 (Applied Biosystems, Foster City, CA, USA). The data for *lysozyme* and *TNF-α* are reported as a ratio of absolute mRNA copy number of each specific gene to the absolute copy number of *β-actin*.

2.6. Immunization trial

One hundred fish from each dietary treatment were transported to the Clear Springs Foods Inc. research laboratory and placed in 378 L fiberglass aquaria receiving ultraviolet light disinfected, single-pass spring water (mean temperature 14.5 °C). Fish were fed their

respective diets *ad libitum* twice daily. One week post-transport, 20 fish were removed from each stock group and anesthetized in 250 mg/L MS-222. Each fish was injected intraperitoneally with 10^6 plaque forming units (PFU) of an infectious hematopoietic necrosis virus (IHNV) cell culture lysate. This virus isolate (039-82) has been previously shown to be significantly less virulent that other IHNV isolates from this area. The virus was isolated, identified, and characterized as previously reported (LaPatra et al., 1994). Each 20 fish group was placed in separate 378 L fiberglass aquaria on a separate water supply.

At 4 weeks post-immunization, 12 immunized fish from each treatment were anesthetized and non-lethally bled. Additionally, five fish from the stock groups that had not been immunized were bled. Blood was obtained by caudal puncture from individual fish after they were anesthetized as previously described. Individual blood samples were placed at 4 °C and allowed to clot overnight. The samples were centrifuged for 10 min at 1600 × *g* and the sera tested using a complement neutralization test for determination of anti-IHNV antibody titers that has been described previously (LaPatra et al., 1993).

2.7. Disease resistance evaluation

To examine disease resistance, fish remaining following sampling and the immunization trial were grouped by dietary treatment. Fish were subsequently challenged with IHNV at two different time points. Approximately twenty fish were experimentally infected at each time point. Briefly, fish were anesthetized and each fish was injected intraperitoneally with 10^6 PFU of IHNV strain 220-90 which has been classified as a highly virulent strain (LaPatra et al., 1994). As a control, 20 additional fish were mock infected by injection with saline at each time point. Fish were monitored for mortality and fed their respective diets for 18 days. A minimum of 20% of the each day's dead fish were examined for virus presence and titer using plaque assay procedures that have been previously described (LaPatra et al., 1989).

2.8. Statistical analysis

Data were analysed for statistical significance by analysis of variance (ANOVA) using SAS (SAS, 1990; version 5.0, SAS Institute Inc., Cary, NC, USA). A significance level of $P<0.05$ was used. Mean tank values were considered units of observation for statistical analysis. Post hoc tests (Tukey's) were used to identify significant differences among treatments. The Wilcoxon rank-sum test and the Mann–Whitney *U*-test were used to make comparisons between antibody titers.

3. Results

3.1. Effect of barley genotype on fish performance

Significant effects of dietary β-glucan on the growth performance of rainbow trout were observed following 3 weeks but not 9 weeks of feeding (Table 2). Following 3 weeks of feeding, growth of fish fed the diet containing the high β-glucan barley was significantly reduced as compared to the control diet. Feed conversion ratio was significantly elevated

Table 2
Growth performance of rainbow trout at 3 and 9 weeks[a]

Diet	3 weeks		9 weeks	
	Weight gain[b]	FCR[c]	Weight gain[b]	FCR[c]
Wheat	139 a	0.67 c	605	0.90
Low β-glucan barley	137 a,b	0.70 b,c	589	0.95
Medium β-glucan barley	143 a	0.73 a,b	622	0.95
High β-glucan barley	115 b	0.77 a	625	0.98
Wheat diet with 2 g/kg MacroGard	137 a,b	0.75 a	577	0.97
Pooled S.E.M.	4.91	0.01	21.09	0.02
P-value[d]	0.0189	0.0008	0.4612	0.1204

[a] Means of three replicate tanks.
[b] Weight gain = ((Final average fish weight−initial average fish weight)/initial average fish weight) × 100.
[c] FCR = feed conversion ratio; (g feed/g gain).
[d] Significance probability associated with the *F*-statistic; values within columns with a common letter do not differ significantly (P>0.05) based on Tukey's.

in groups fed the high β-glucan barley or the commercially available yeast β-glucan diets when compared to fish fed the wheat control diet or the low β-glucan barley diets. Following 9 weeks of feeding, no significant differences among dietary treatments were observed for growth or feed conversion ratio (Table 2).

3.2. Effect of barley genotype on whole-body proximate composition

Diet had only minor effects on whole body proximate composition (Table 3). Fish fed the wheat control diet or the high β-glucan barley diet had significantly higher moisture content than fish fed the low β-glucan barley diet following 3 weeks of feeding. Following 9 weeks of feeding, no significant differences among dietary treatments were observed for whole body protein, lipid, ash or energy.

3.3. Effect of barley genotype on immune responses

Diet had no significant effect on respiratory burst responses following 3 or 9 weeks of feeding (data not shown). Similarly, *lysozyme* and *TNF-α* mRNA expression displayed a high degree of variability and no statistically significant differences were observed for either gene following feeding of their respective diets for 3 or 9 weeks.

IHNV neutralizing antibody titers of immunized rainbow trout displayed effects of diet (Table 4). Fish fed diets containing the medium β-glucan barley displayed significantly higher antibody responses following vaccination than fish fed the commercial yeast β-glucan supplemented diet.

3.4. Effect of barley genotype y on survival following IHNV challenge

Barley genotype did alter survival following IHNV challenge (Table 5). Fish fed diets containing the average or high β-glucan containing barley genotypes had survival similar

Table 3
Proximate composition[a] of rainbow trout fed experimental diets for 3 and 9 weeks

Diet	3 weeks					9 weeks				
	Moisture	Protein	Lipid	Ash	Gross energy	Moisture	Protein	Lipid	Ash	Gross energy
Wheat	743 z	157	81	19	27,415	695	172	122	16	29,434
Low β-glucan barley	728 y	165	94	21	28,062	696	171	116	18	30,288
Medium β-glucan barley	737 yz	160	96	19	28,127	691	171	124	18	30,289
High β-glucan barley	750 z	158	79	22	27,468	700	169	114	20	30,240
Wheat diet with 2 g/kg MacroGard	737 yz	160	88	21	27,468	701	172	113	17	30,416
Pooled S.E.M.	2.93	2.77	4.27	0.93	394	6.21	2.61	7.28	1.30	279
P-value	0.0040	0.3059	0.0672	0.2184	0.5976	0.8035	0.8912	0.7499	0.2587	0.1662

[a] Whole body moisture, protein, lipid and ash (g/kg) and energy (MJ/kg) means of three replicate tanks per treatment.

Table 4
IHNV neutralizing antibody titers in immunized rainbow trout that had previously been fed the experimental diets for 9 weeks[a]

Individual fish	Wheat	ow β-glucan barley	Medium β-glucan barley[b]	High β-glucan barley	Wheat with 2 g/kg MacroGard
1	<20	<20	20	<20	<20
2	<20	<20	20	<20	<20
3	<20	20	80	<20	<20
4	<20	40	80	20	<20
5	<20	40	80	20	20
6	<20	80	80	80	20
7	20	80	>160	80	20
8	20	>160	>160	>160	40
9	80	>160	>160	>160	40
10	>160	>160	>160	>160	80
11	>160	>160	>160	>160	80
12	>160	>160	>160	>160	80

[a] Values for 12 replicate fish with negligible titers prior to immunization.
[b] Fish fed diets containing the medium β-glucan barley displayed significantly higher antibody responses following vaccination than fish fed the commercial yeast β-glucan supplemented diet by Wilcoxon Rank-sum and Mann–Whitney *U*-test.

to those fed the commercial yeast β-glucan supplemented diet and higher than trout fed the wheat control diet following IHNV challenge. Of the fish that died during the challenges and were examined for IHNV presence, 96% (150/157) were positive for the virus and the mean concentration detected was 3×10^6 PFU/g (range, 10^2 to $>2 \times 10^7$ PFU/g) indicating that the cause of mortality in the study was IHNV.

4. Discussion

The growth of fish is known to be affected by both the inclusion level of dietary carbohydrate and degree of carbohydrate complexity (Wilson and Poe, 1987; Hung and Storebakken, 1994). In general coldwater fish, like the rainbow trout, have a limited ability to utilize carbohydrates as energy sources (Wilson, 1994) and for that reason most commercial diet formulations for salmonids restrict dietary carbohydrate inclusion to less than 300 g/kg, with wheat or gelatinized corn starch being the most commonly utilized sources. In the present study, substituting barley for the wheat portion of a practical-type diet did not substantially alter weight gain or proximate composition of rainbow trout suggesting that both cereal grains were equally utilized in the present study even though barley contains less starch and more dietary fiber than wheat. These findings are supported by the work of Cheng and Hardy (2003) in which it was shown that barley and wheat had comparable digestible energy and protein values.

In contrast, positive effects on disease resistance were observed following substitution of barley for the wheat component in a practical type trout diet. Fish fed diets containing the average or high β-glucan containing barley genotypes had survival similar to those fed the commercial yeast β-glucan supplemented diet and higher than trout fed the wheat control

Table 5
Survival of rainbow trout fed experimental diets following intraperitoneal injection challenge with IHNV[a]

Diet	Challenge 1			Challenge 2			Summary[b]
	No. of fish	No. of morts	% survival	No. of fish	No. of morts	% survival	Average % survival
Wheat	20	17	15	21	15	29	22 y
Low β-glucan barley19	19	11	42	19	10	47	45 yz
Medium β-glucan barley	17	6	65	20	7	65	65 z
High β-glucan barley	19	10	47	20	9	55	51 z
Wheat diet with 2 g/kg MacroGard	18	5	70	20	9	45	58 z

[a] Means of two challenges.
[b] Values within columns with a common letter do not differ significantly at $P \leq 0.05$ based on.

diet following IHNV challenge. LaPatra et al. (1998) reported previously that fish injected with the same commercial yeast β-glucan exhibited lower mortalities after waterborne exposure to IHNV and lower antibody titers when compared to sham-challenged fish. These authors hypothesized that glucan-enhanced macrophage activity and/or other non-specific defense mechanisms minimized the humoral response to IHNV.

Numerous studies have addressed the immunostimulating potential of β-glucan in fish (for review see Robertsen, 1999; Sakai, 1999; Sealey, 2000). Branched chain β 1,3 1,6 linked glucans found in yeast have been the primary focus of research in fish, although some studies indicate that the more linear β 1,3 1,4 linked glucans isolated from barley are effective immunostimulants (Jeney and Anderson, 1993; Wang and Wang, 1996a,b). Previous protocols shown to enhance immune response and disease resistance in rainbow trout indicate that 1–2 g/kg glucan in the diet for 1–4 weeks was effective (Siwicki et al., 1994). In the current study a barley β-glucan content of approximately three times the yeast β-glucan levels was necessary to produce the same level of disease resistance; increased dosage requirements have been previously reported in order to obtain similar responses utilizing the more linear β 1,3 1,4 linked glucans. However, in the current study no detrimental effects of feeding elevated levels of glucans for periods longer than the currently recommended 2-week period on immune responses and disease resistance were observed in contrast to previous reports (Jeney et al., 1997).

Macrophage activation plays a central role in the mechanism of β-glucan-mediated immunostimulation in humans (Czop, 1986; Goldman, 1988) and in fish (Jorgensen and Robertsen, 1995; Robertsen, 1999). To examine macrophage activation state in fish, respiratory burst response assays have most often been employed. In this manner, several studies have previously shown that purified yeast and barley β-glucans have a potent ability to up-regulate macrophage function from various fish species *in vitro* (Engstad and Robertsen, 1993; Jorgensen et al., 1993; Secombes, 1994; Jorgensen and Robertsen, 1995) and *in vivo* (Wang and Wang, 1996a,b). In the present study we were unable to correlate the previously observed up-regulations in respiratory burst with increased dietary β-glucan content likely due to the high variability observed among fish.

Recently sequenced immune-related genes have also been shown to be useful tools for the examination of immune function and predictors of disease resistance. Specifically, studies in mammals indicate a molecular mechanism by which NF-κB induction (Adams et al., 1997; Young et al., 2001) and the temporal release of cytokines including tumor necrosis factor alpha (TNF-α) and interleukin 1 beta (Il-1β) from macrophages (Czop and Austen, 1985; Abel and Czop, 1992) occur following β-glucan receptor-specific binding. In contrast no significant transcriptional changes in *TNF-α* expression were observed in the current study following feeding of the various diets for 3 or 9 weeks. Similarly, *lysozyme* expression was not significantly altered by diet in the current study, although other studies (Paulsen et al., 2001, 2003) have demonstrated substantial increases in lysozyme activity following β-glucan treatment.

The lack of correlation between the immune response factors examined in this study and dietary β-glucan content could indicate that the observed increases in disease resistance are not due to β-glucan binding and subsequent increased macrophage activation or that sampling times were not sufficient to detect transcriptional changes in the examined immune response factors. In support of this latter hypothesis, not all mammalian studies

have been able to detect transcriptional changes in *TNF-α* following glucan binding (Tran Thi et al., 1995). One theory proposed to explain the lack of transcriptional response in the mammalian study by Tran Thi et al. (1995) was a lack of dosage and sampling time optimization for the examined glucan (Young et al., 2001). Thus the limited sampling protocol in the current study may have reduced our ability to detect dietary induced changes. Alternatively, the observed dietary dependent increases in antibody titers and disease resistance could also be related to a more general prebiotic type effect of increasing non-starch polysaccharides content on fish heath or an altogether different unspecified component present in the various barley varieties.

5. Conclusions

Our results indicate that ground barley from select genotypes can be substituted for wheat without significant detrimental effects on production efficiency while possibly increasing resistance to viral pathogens. Although the underlying immunostimulatory mechanisms remain undefined, the current study provides supporting evidence that β-glucans endogenous in barley can increase disease resistance in rainbow trout.

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